UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF A PLANT REGENERATION SYSTEM AND ANALYSIS OF 101 HEAT SHOCK PROTEIN IN STRAWBERRY cv. CAMAROSA FOLLOWING GENE BOMBARDMENT

FATEMEH HADDADI

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By

FATEMEH HADDADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

2009

In The Name of Allah
The Most Gracious, the Most Merciful

Specially dedicated to:

My beloved
Hossein

And

My kind parents
Habib and Parvin

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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February 2009

Chairperson: Associate Professor Maheran Abdul Aziz, PhD

Faculty : Agriculture

The aims of this study were to develop *in vitro* regeneration system and to confirm the transient expression of HSP101 gene via protein analysis in strawberry cv. Camarosa.

In the *in vitro* study, two types of explants which were shoot tips derived from runner tips and leaves were used. Different types of cytokinins such as BAP, TDZ and zeatin at different concentrations were assessed for shoot induction, while the auxins IBA and NAA also at different concentration were used in the root induction experiment. The experiments were conducted in a Randomized Complete Block Design (RCBD).

In the shoot induction experiment using shoot tips cultured on different concentrations and combinations of TDZ and BAP, MS medium

supplemented with 4 µM BAP in combination with 2 µM TDZ was optimum for strawberry shoot proliferation. In the shoot induction experiment from shoot tips using zeatin, the highest percentage of explant producing shoots and number of shoots formed per explant were obtained on MS medium containing 4 µM zeatin. High frequency of shoot regeneration from strawberry leaves using different concentrations and combinations of BAP and TDZ was achieved on MS medium containing 4 µM TDZ, without BAP. In the rooting study, MS medium containing 1 µM NAA, MS medium containing 1 µM IBA and MS medium without auxins, were most suitable in inducing the highest number of roots per explant, highest percentage of root formation and the longest root, respectively.

Biolistic method of gene transfer has the advantage of allowing a fast and rapid analysis, and was therefore selected for transient expression of HSP101 gene in strawberry via protein analysis. In this study, *in vitro* leaf explants of strawberry were used. Transient gene expression assays of the *At*HSP101 gene showed that this gene can be transiently expressed in strawberry plants. An additional faint protein band of approximately 100 kD was observed on SDS polyacrylamide gel electrophoresis after bombardment of the leaf explant with plasmid, which most probably corresponded to the HSP101 encoded product. In the study on total protein assay using Bradford method, the amount of total protein after bombardment of leaf explant with plasmid containing

HSP101 gene increased in comparison with bombardment without plasmid and with non bombarded explants. This result also confirmed that this gene can be transiently expressed in strawberry plants. Therefore by using the regeneration protocol obtained in this study and HSP101 gene which can be transiently expressed, genetic engineering of strawberry cv. Camarosa for heat tolerance can be achieved.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

PEMBENTUKAN SISTEM REGENERASI TUMBUHAN DAN ANALISA PROTEIN KEJUTAN HABA 101 KEATAS STRAWBERI cv. CAMAROSA SELEPAS PEMBEDILAN GEN

Oleh

FATEMEH HADDADI

Februari 2009

Pengerusi : Professor Madya Maheran Abdul Aziz, PhD

Fakulti : Pertanian

Tujuan kajian ini adalah untuk membangunkan sistem regenerasi secara *in vitro* dan untuk memastikan pengekspresan transien gen HSP101 melalui analisa protein pada strawberi cv. Camarosa.

Dalam kajian *in vitro*, dua jenis eksplan iaitu tunas hujung daripada hujung 'runner' dan daun digunakan. Jenis sitokinin yang berbeza seperti BAP, TDZ dan zeatin pada kepekatan yang berbeza telah diuji untuk induksi tunas, manakala auksin IBA dan NAA juga pada kepekatan yang berbeza digunakan untuk kajian induksi akar. Eksperimen dilaksanakan dalam Rekabentuk Blok Berawak Penuh (RCBD).

Dalam kajian induksi tunas menggunakan tunas hujung yang dikultur pada kepekatan dan kombinasi TDZ dan BAP yang berbeza, medium MS yang ditambahkan dengan 4 μM BAP dengan kombinasi 2 μM TDZ adalah optima untuk penggandaan tunas strawberi. Dalam kajian induksi tunas daripada tunas hujung menggunakan zeatin, peratus eksplan yang mengeluarkan tunas dan bilangan tunas terbentuk per eksplan yang paling tinggi diperoleh pade medium MS yang mengandungi 4 μM zeatin. Frekuensi regenerasi tunas yang tinggi daripada daun strawberi diperoleh pada medium MS yang mengandungi 4 μM TDZ tanpa BAP. Dalam kajian pengakaran, medium MS yang mengandungi 1 μM NAA, medium MS yang mengandungi 1 μM IBA dan medium MS tanpa auksin, masing-masing adalah sangat sesuai untuk induksi bilangan akar per eksplan paling tinggi, peratusan pembentukan akar paling tinggi dan akar yang terpanjang.

Pemindahan gen melalui kaedah biolistik mempunyai kelebihan ke arah analisis yang cepat dan segera, maka dipilih untuk pengekspresan transien gen HSP101 di dalam strawberi melalui analisis protein. Dalam kajian ini, eksplan daun *in vitro* strawberi telah digunakan. Esei pengekspresan gen transien bagi gen *At*HSP 101 menunjukkan bahawa gen ini dapat diekspresikan dalam tumbuhan strawberi. Tambahan jalur protein saiz 100 kD yang samar dilihat pada elektroforesis gel poliakrilamid selepas pembedilan eksplan daun, yang

berkemungkinan besar produk pengkodan HSP101. Dalam kajian esei protein total menggunakan kaedah Bradford, kandungan protein total selepas pembedilan eksplan daun dengan plasmid yang mengandungi gen HSP101 meningkat berbanding dengan pembedilan tanpa plasmid dan dengan eksplan tanpa pembedilan. Hasil ini juga memastikan bahawa gen ini dapat diekspresikan secara transien di dalam tumbuhan strawberi. Oleh itu, dengan menggunakan protokol regenerasi yang telah diperoleh dan gen HSP101 yang boleh diekspresi, kejuruteraan genetik strawberi cv. Camarosa untuk toleransi terhadap haba boleh dicapai.

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I certify that an Examination Committee has met on November 2008 to conduct the final examination of Fatemeh Haddadi on her Master of Science thesis entitled “Development of a Plant Regeneration System and Analysis of 101 Heat Shock Protein in Strawberry cv. Camarosa Following Gene Bombardment” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the Master Science.

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Date: 8 June 2009

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged .I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

FATEMEH HADDADI

NAME OF STUDENT
Date:

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
*At*HSP101	*Arabidopsis thaliana* heat shock protein 101
BA	N6-benzyladenine
BAP	6-benzylaminopurine
bp	base pairs
BSAA	3-benzo[b] selenienyl acetic acid
CaMV	cauliflower mosaic virus
$CaCl_2$	calcium chloride
cDNA	complementary DNA
$CoCl_2\,6H_2O$	cobalt chloride 6-water
$CuSO_4\,5H_2O$	cuprum sulfate 5-water
cv.	cultivar
$CoCl_2\,6H_2O$	cobalt chloride 6-water
DNA	deoxy ribonucleic acid
DNMRT	duncan new multiple range test
EDTA	ethylene diamine tetra acetic acid
E.coli	*Escherichia coli*
et al.	et alia
FW	fresh weight
GFP	green fluorescent protein
GUS	β-glucuronidase
HCl	hydrochloric acid

HSP	heat shock protein
IAA	indole-3-acetic acid
IBA	Indol-3-Butyricacid
Kb	kilobases
kD	kilodaltons
KH_2PO_4	potassium dihydrogen phosphate
KI	potassium iodide
KNO_3	potassium nitrate
KOH	potassium hydroxide
LB	Luria Bertani medium
$MgSO_4$	manganese sulfate 4-water
$\mu mol\ m^{-2}\ s^{-1}$	micromole per square meter per second
MS	Murashige and Skoog
NAA	a-naphthaleneacetic acid
NaCl	sodium hydroxide
$Na_2MoO_4\ 2H_2O$	natrium molybdate 2-water
NH_4NO_3	ammonium nitrate
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PGR	plant growth regulator
pH	$-\log (H^+)$
RCBD	randomized complete block design
RNAase	ribonuclease
rpm	revolutions per minute

SDS	sodium dodecyl sulphate
UV	ultraviolet (light)
v/v	volume to volume
w/v	weight to volume
$ZnSO_4$ $7H_2O$	zinc sulfate 7-water
2,4-D	2,4-dichlorophenoxyacetic acid

CHAPTER 1

INTRODUCTION

The strawberry belongs to the *Rosaceae* family as the third economically important cultivated crop (Oosumi *et al*., 2006). The family also includes raspberry and blackberry. Strawberries are of the genus *Fragaria*. There are more than 20 named species and 600 strawberry cultivars found today and they stem from five or six original wild species. The most common type of strawberries grown commercially are cultivars of the Garden strawberry (*Fragaria* × *ananassa*). The strawberry fruits are rich in bioactive phytochemicals, especially phenolic compounds with high antioxidant capacity, and can be beneficial to human health when they are consumed as part of the daily diet (Hannum, 2004).

Yearly strawberry production varies from 500,000 in Asian countries to 1 million tons in European countries (Gruchala *et al*., 2004). At present 71 countries in the world are producing strawberry on 506,000 acres (Sakila *et al*., 2007). The production of this valued fruit is mainly concentrated in North America. The United States is the largest producer of strawberries, accounting for over a quarter of total world production and the second largest total harvested area after Poland (FAOSTAT, 2007).